Understanding Water Activity for Reduced Microbial Testing Using USP Method <1112>

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Abstract

Water is recognized as being very important, if not critical, to the chemical, physical, and microbiological stability of most pharmaceuticals. Controlling the water within a product, by chemically or structurally binding it or else through some method of drying has long been used in the pharmaceutical industry. Water activity is a measure of the energy status of water in a product and is reduced through chemical binding or drying. It's not the amount of water, but rather the water activity that plays a critical role in the microbiological, chemical, and physical stability of pharmaceutical dosage formulations and ingredients. Knowledge of the water activity of pharmaceuticals is essential to obtain a dosage formulation with optimal shelf life properties.

Introduction

Water activity is defined as the ratio of the vapor pressure of water in a material (p) to the vapor pressure of pure water (po) at the same temperature. Relative humidity of air is defined as the ratio of the vapor pressure of air to its saturation vapor pressure. When vapor and temperature equilibrium are obtained, the water activity of the sample is equal to the relative humidity of air surrounding the sample in a sealed measurement chamber. Multiplication of water activity by 100 gives the equilibrium relative humidity (ERH) in percent.

$$a_w = p/p_o = ERH\ (\%) / 100$$

As described by the above equation, water activity is a ratio of vapor pressures and thus has no units. It ranges from 0.0 a_w (bone dry) to 1.0 a_w (pure water). There are several factors (osmotic, matrix, and capillary) that control water activity in a system. It is a combination of these factors in a product that reduces the energy of the water and thus reduces the vapor pressure above the sample as compared to pure water. Water activity is a measure of how tightly water is "bound" and related to the work required to remove water from the system. Due to varying degrees of osmotic and matrix interactions, water activity describes the continuum of energy states of the water in a system rather than a static "boundness". Water that is "bound" should not be thought of a totally immobilized.

Microbial and chemical processes are related to water's "bound" energy status in a fundamental way. Since moisture content only provides information about the amount of water and not the availability or "boundness" of water, it is unreliable for determining susceptibility to microbial growth. Because water is present in varying energy states, analytical methods that attempt to measure total moisture in samples don't always agree or relate to safety and quality. For example, a product may contain a relatively large percentage of moisture, but if the water is chemically "bound" with the addition of humectants or solutes, such as salts, sugars, or polyols, the water is biologically unusable for the microbial growth processes. The water activity concept has served microbiologists and food technologists for decades and is the most commonly used criterion for food safety and quality, however water activity has not been widely adopted in the pharmaceutical industry.

Now there is a published USP (United States Pharmacopeial) Method <1112> that utilizes water activity. USP Method <1112> Microbiological Attributes of Non-sterile Pharmaceutical Products – Application of Water Activity Determination provides guidance on the influence of water activity as it pertains to the susceptibility of a product formulation to microbial contamination. Knowledge of the behavior of

microorganisms in pharmaceutical products at different a_w levels is essential to effectively utilize USP method <1112>. The chapter discusses the potential for improving product preservation by maintaining low water activity. The determination of the water activity of non-sterile pharmaceutical dosage forms aids in the following:

- Optimizing product formulations to improve antimicrobial effectiveness of preservative systems
- Reducing the degradation of active pharmaceutical ingredients within product formulations susceptible to chemical hydrolysis
- Reducing the susceptibility of formulations (especially liquids, ointments, lotions, and creams) to microbial contamination
- Providing a tool for the rationale for reducing the frequency of microbial limit testing and screening for objectionable microorganisms for product release and stability testing using methods contained in the general test chapter MicrobialLimit Tests <61>

Materials and Methods

Minimum water activity values for growth were obtained from a survey of literature values. AquaLab Series 3TE (Decagon Devices, Inc., Pullman WA) was used for all testing of pharmaceutical and over-the-counter (OTC) drug products.

Results

Table 1 lists the minimum water activity level for growth of USP-specified and objectionable microorganisms for pharmaceutical products. The lowest a_w at which the vast majority of pathogenic bacteria will grow is about 0.91. The only exception to this is Staphylococcus aureus, which will grow at a water activity of 0.86 under aerobic conditions. The minimum water activity level for growth of molds and yeast is 0.70 and is lower than that for bacteria. The lowest level for any microbial growth is 0.61.

Microorganisms require a certain amount of “free” water to support growth (Table 1). Control of the water activity in a pharmaceutical product can be used to inhibit microbial growth. Water activity controls all aspects of microbial growth.

Table 1 Minimum Water Activity Level for Growth

Microorganism	Minimum Water Activity for Growth
Pseudomonas aeruginosa *Clostridium botulinum, Type E*	0.97
Bacillus cereus *Clostridium botulinum, Type A, B* *Escherichia coli* *Salmonella spp.* *Clostridium perfringens* *Burkholderia cepacia* *Klebsiella* *Lactobacillus viridescens* *Pseudomonas aeruginosa*	0.95
Enterobacter aerogenes *Pseudomonas fluorescens*	0.94
Micrococcus lysodekticus *Rhyzopus nigricans*	0.93
Listeria monocytogenes *Mucor plumbeus* *Rhodotorula mucilaninosa*	0.92
Serratia marcesens *Plesiomonas shigelloides* *Shigella spp.* *Vibrio chlorea* *Vibrio parahaemolyticus* *Yersina enterocolitica* *Yersina pseudotuberculosis* *Aeromonas caviae* *Aeromonas hydrophilia* *Aeromonas sobria*	0.91
Bacillus subtilis *Staphylococcus aureus (anaerobic)* *Saccharomyces cerevisiae*	0.91
Candida	0.88
Staphylococcus aureus (aerobic)	0.87
Paecilomyces variotti	0.84
Penicillium chrysogenum	0.83
Aspergillus fumigatus	0.82
Penicillium glabrum	0.81
Aspergillus flavus	0.78
Aspergillus niger	0.77
Halobacterium halobium (halophilic bacterium)	0.75
Zygosachharomyces rouxii (osmophilic yeast)	0.62
Xeromyces bisporus (xerophilic fungi)	0.61
No Microbial Proliferation	<0.60

Table 1 lists the minimum water activity level for growth of different microorganisms. At water activity levels above minimal growth limits, water activity lengthens the lag phase, lowers the log growth phase, and reduces the stationary phase number of organisms. If the microorganism produces a toxin, then the toxin production will stop at a higher water activity level than growth inhibition. Low water activity also prevents spore germination if spores are present in the product. Water activity is not a kill step. Some organisms can survive for a period of time at lower water activity levels. These organisms are not actively growing, but are waiting for conditions to change (i.e. increased a_w). Microorganisms do die at slow rates at the lower water activity levels. Thus, it is important to know or control the water activity level of pharmaceuticals.

Reduced water activity will greatly assist in the prevention of microbial contamination of pharmaceutical products, and the formulation, manufacturing, and testing of non-sterile dosage forms should reflect this parameter. When formulating drug products, the water activity should be evaluated so that the product may be self-preserving.

To lower water activity of a product, one can remove water, add ingredients, or lower the temperature. Adding ingredients such as salt, sugars, glycols, glycerin or amino acids will result in a formulation with a lower water activity that discourages the proliferation of microorganisms in the product. These added ingredients cause some of the “free” water to be “bound” and thus unavailable to the microorganisms for growth. However, there is a limit to how much of these additives one can add before it effects the taste and texture of the product.

Since different products will have different concentrations of water activity reducing ingredients and different amounts of water, each product type will have a unique water activity.

Table 2 lists the water activity values or ranges of common pharmaceutical and OTC drug products.

Table 2 Water Activity Values of Typical Pharmaceutical and OTC Drug Products

Main Category, **Subcategory**, Item	a_w Value or Range	Source
Pharmaceuticals		
Analgesic	0.401	1
Analgesic (gelatin capsules) liquid	0.53	1
Analgesic (gelatin capsules) gelatin	0.533	1
Anti-allergic	0.443	1
Antibiotic pills (cefacilin)	0.441	1
Anti-migraine, pills	0.386	1
Anti-inflammatory Cream	0.852	1
Anti-inflammatory Ointment	0.975	1
Anti-inflammatory Suspension	0.87	2
Antimicotic Cream	0.95	1
Anti-micotic Powder	0.537	1
Aspirin	0.44	1
Citrobioflavonoide & vit. C syrup	0.801	1
Cough Drop, Liquid Center	0.4	3
Cough Suppressant	0.89	3
Cough Syrup	0.912-0.965	1
Decongestant/Antihistamine (liquid-filled capsule)	0.45	3
Epileptic Syrup	0.835	1
Lactulose Syrup (laxative)	0.823	1
Laxative	0.927	1
Menstrual pain, pills	0.459	1
Mucolitic elixir	0.904	1
Neurotonic Syrup	0.935	1
Potassium gluconate, elixir	0.926	1
Rectal Suppositories	0.29	3
Tonic Syrup	0.95	1
Vaginal Suppositories	0.3	3
Vitamin C tablets	0.33	1
Vitamin, multivitamin tablet	0.3	3
Ointments/Creams		
Bactericidal cream	0.841	1
Canker Sore Gel, oral	0.86	3

Cicatrizant cream	0.978	1
Cream for Dermatitus	0.951-0.952	1
Gel anti-inflammatory (topical use)	0.942	1
Rectal Cream	0.97	1
Rectal Ointment	0.26	1
Vaginal anti-micotic cream	0.982	1
Consumer Products		
Hair Products		
Hair gel	0.982	1
Shampoo	0.982-0.987	1
Lotions		
Body Cream	0.972-0.983	1
Deodorant gel bar	0.984	1
Lip Balm, topical/oral	0.36	3
Sun Blocker	0.940-0.981	1
Soap		
Soap, Creamed	0.567	1
Soap, Regular	0.740-0.757	1
Soap, with glycerin	0.740-0.757	1
Soap, with glycerin and lanolin	0.856	1
Toothpaste		
Toothpaste	0.585-0.984	1

Conclusion

Table 2 can be used to establish a microbial limit testing strategies for typical pharmaceutical and OTC drug products based on water activity. Pharmaceutical drug products with water activities well below 0.75, i.e., direct compression tablets, liquid-filled capsules, nonaqueous liquid products, ointments, and other listed in Table 2, would be excellent candidates for reduced microbial limit testing. This is especially true when pharmaceutical products are made from ingredients of good microbial quality, in manufacturing environments that do not foster microbial contamination, by processes that inherently reduce the microbial content, and in manufacturing sites that have an established testing history of low bioburden associated with their products.

References:

1. Decagon Devices in-house testing, Pullman Washington. a_w values were obtained using Decagon AquaLab Water Activity Meters Series 3TE (chilled mirror).

2. Labuza TP. 1993. Water activity: theory, management, and applications. AACC Water Activity Course. February 16-19, 1993, St Paul, MN.

3. Friedel RR and Cundell AM. 1998. The application of water activity measurement to the microbiological attributes testing of non-sterile over-the-counter drug products. Pharmacopeial Forum 24:6087-6090.

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